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Note

Low-energy collision-activated dissociation electrospray ionization tandem mass spectrometric analysis of *Sinorhizobial* succinoglycan monomers

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ABSTRACT

Succinoglycan monomers (M1, M2, and M3) are octasaccharides with acetyl, pyruvyl, and/or succinyl groups as substituents derived from *Sinorhizobium meliloti* 1021. The dissociation patterns of the octasaccharides caused by low-energy collision-activated dissociation (CAD) were investigated using triple quadrupole tandem mass spectrometry (MS) equipped with an electrospray ionization (ESI) source with increasing collision energy (CE) in negative ion mode. None of the succinoglycan monomers were fragmented at a CE of −25 eV. When the CE was applied to −50 or −70 eV, the loss of the terminal Gal residue and/or the succinyl group of the monomers was observed in the product ion scan mode. Interestingly, the acetyl and the pyruvyl groups in the succinoglycan monomers were not lost even when a CE of −70 eV was applied, indicating that the substituents are more stable than the succinyl group in the octasaccharides.

The symbiotically important exopolysaccharide, succinoglycan, is necessary for successful invasion of alfalfa root nodules by *Sinorhizobium meliloti* Rm1021.[1–3] *S. meliloti* Rm1021 synthesizes both high- and low-molecular weight succinoglycan (LMWS).[4,5] Among them, the LMWS has been reported to promote the formation of nitrogen-fixing nodules.[4,6]

From a structural point of view, the high-molecular weight succinoglycan (HMWS) originating from *S. meliloti* Rm1021 is a biopolymer composed of an octasaccharide repeating unit modified with acetyl, pyruvyl, and succinyl substituents.[7–10] A structural characterization of the octasaccharide repeating unit of HMWS, in which succinoglycan samples were hydrolyzed to the octasaccharide repeating units with a specific succinoglycan depolymerase from *Cytophaga arvensicola*, was performed with NMR spectroscopy[11] and MS.[12] The octasaccharide repeating unit prepared through enzymatic degradation was shown to contain one acetyl, one pyruvyl, and one succinyl group as substituents.

Some chromatographic techniques and NMR analyses also revealed that the LMWS consisted of monomers, dimers, and trimers, and there is considerable heterogeneity in the monomer, the dimer, and the trimer in terms of noncarbohydrate substitutents.[13] The distributions of succinyl groups within the molecules of the dimer and the trimer showed more random succinylation patterns than those of the monomer, indicating that each species of the dimers or the trimers is difficult to characterize in detail.[13] Among the LMWS, the monomers are classified into M1, M2, and M3, depending on their substituents, as shown in Figure 1. The monomer, M1, is an octasaccharide with one acetyl and one pyruvyl group, and M2 is an octasaccharide with one acetyl, one pyruvyl, and one succinyl group, and M3 is an octasaccharide with one acetyl group, one pyruvyl group, and two succinyl groups.[13] It has been recently reported that the monomers have potential to be chiral

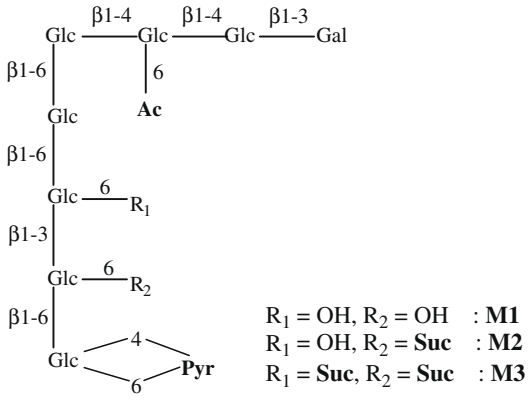

Figure 1. The chemical structure of the monomers (Glc: glucose; Gal: galactose; Ac: acetyl; Suc: succinyl; Pyr: pyruvyl group), based on previous reports.[7,10]

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Table 1
CAD fragment ions and their corresponding *m/z* observed from $[M-H]^-$ ions of the succinoglycan monomers with increasing CE

CE (eV)	Succinoglycan monomers		
	M1	M2	M3
−25	—	—	—
−50	—	$[M2-H-Suc]^-$, *m/z* 1425	$[M3-H-Suc]^-$, *m/z* 1525
		$[M2-H-Gal]^-$, *m/z* 1363	$[M3-H-Gal]^-$, *m/z* 1463
−70	$[M1-H-Gal]^-$, *m/z* 1263	$[M2-H-Suc]^-$, *m/z* 1425	$[M3-H-Suc]^-$, *m/z* 1525
		$[M2-H-Gal]^-$, *m/z* 1363	$[M3-H-Gal]^-$, *m/z* 1463
		$[M2-H-Gal-Suc]^-$, *m/z* 1263	$[M3-H-Gal-Suc]^-$, *m/z* 1363
			$[M3-H-Gal-2Suc]^-$, *m/z* 1263

selectors for enantiomeric resolution,[14] sensing electrodes for the detection of quercetin,[15] and organic catalysts in the Strecker reaction,[16] based on their unique physico-chemical properties.

In a previous report,[13] the structures of the monomers were characterized through NMR spectroscopic and compositional analyses. To date, however, the decomposition patterns of the monomers, M1, M2, and M3 caused by low-energy CAD has not been reported. In this study, we investigated the low-energy CAD patterns of the monomers, M1, M2, and M3, which are isolated from *S. meliloti* Rm1021 in product ion scan mode using ESI tandem MS.

After purification of the octasaccharides using some chromatographic techniques, NMR analysis of the purified monomers was carried out, and the data matched those reported previously.[13,16]

The deprotonated ions of the monomers in the negative ion mode were observed in the first quadruple (Q1) as parent ions, where ions at *m/z* 1425, 1525, and 1625 correspond to the molecular masses $[M1-H]^-$, $[M2-H]^-$, and $[M3-H]^-$ of the monomer M1, M2, and M3, respectively. Each ion of the monomers was subjected to CAD in the second quadruple (Q2) using product ion scan mode and then the low-energy CAD tandem mass spectra of the M1, M2, and M3 were obtained, and the spectra were different depending on the octasaccharides and applied CEs.

None of the octasaccharides were fragmented at a CE of −25 eV, as given in Table 1. The M1 was not fragmented, even at a CE of

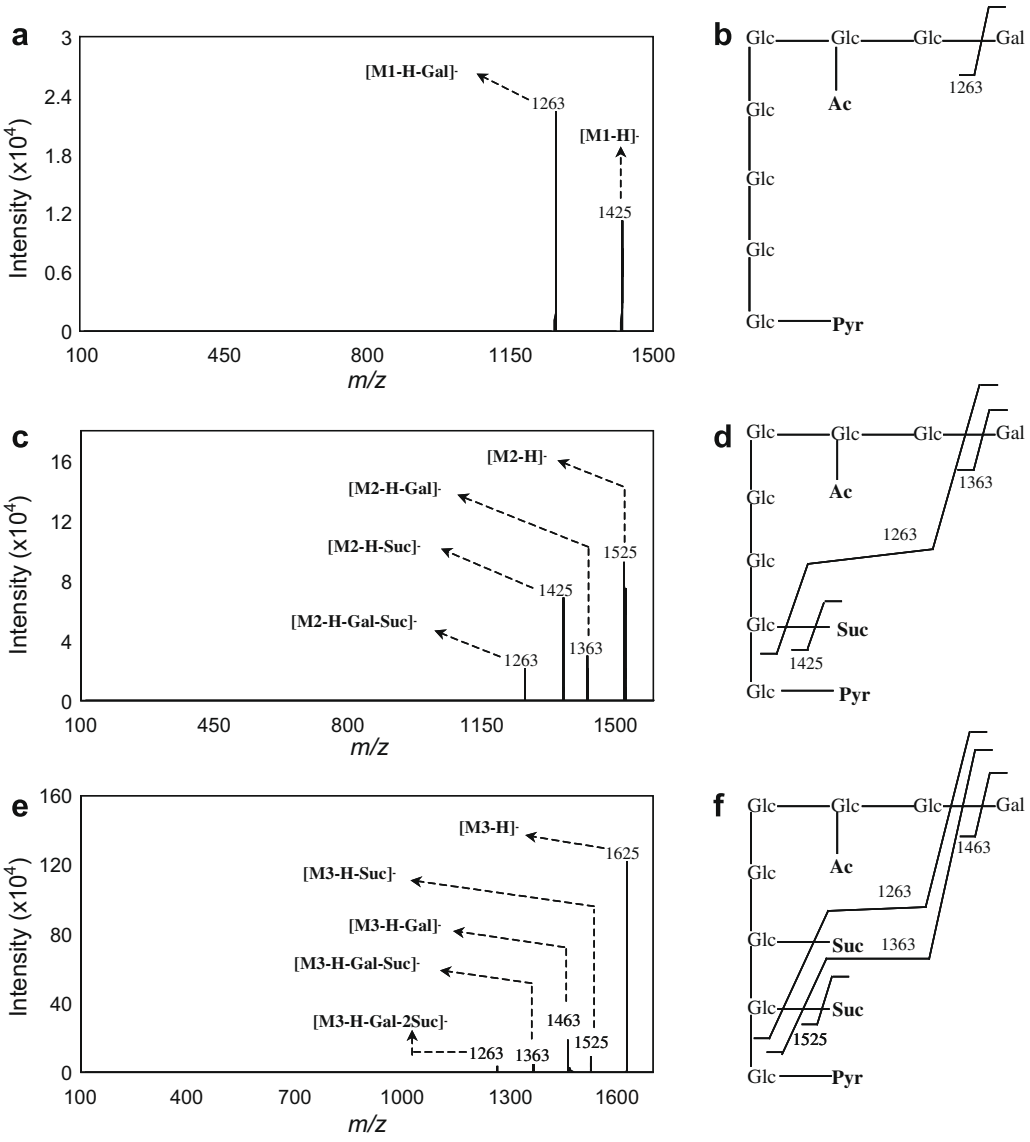

Figure 2. ESI-CAD mass spectra of the monomers (a) M1, (c) M2, and (e) M3 at a CE of −70 eV, and scheme illustrating fragment masses of the cleavages of the glycosidic bond and the succinyl substituents observed in the CAD spectrum of the octasaccharides, (b) M1, (d) M2, and (f) M3.

−50 eV but Gal residue from the reducing end of the octamer was only released when the CE was applied at −70 eV, and then the resulting ion at *m/z* 1263 corresponded to $[M1-H-Gal]^-$ (Fig. 2a and b).

CAD of the M2 led to the loss of succinyl substituent as well as Gal residue within its backbone structure at a CE of −50 eV, and then the fragmented ions were observed at *m/z* 1425 and 1363 corresponding to $[M2-H-Suc]^-$ and $[M2-H-Gal]^-$, respectively (Table 1). At a CE of −70 eV, the ion at *m/z* 1263 corresponding to [M2−H−Gal-Suc] was additionally observed (Fig. 2c and d). These results indicate the cleavage of the glycosidic bond between Glc at the second residue and Gal at the first residue from the reducing end and the release of succinyl group linked with Glc at the seventh residue from the reducing end.

As in the case of M2, the CAD of the M3 also led to the release of the succinyl residue and the terminal Gal residue at a CE of −50 eV and then the resulting ions were observed at *m/z* 1525 and 1463, corresponding to $[M3-H-Suc]^-$ and $[M3-H-Gal]^-$, respectively (Table 1). At a CE of −70 eV, the ion at *m/z* 1263 corresponding to the $[M3-H-Gal\text{-}2Suc]^-$ resulting from the release of succinyl substituent linked with Glc at the sixth residue from the reducing end, including the $[M3-H-Suc]^-$ ion at *m/z* 1525, the $[M3-H-Gal]^-$ ion at *m/z* 1463, and the [M3−H−Gal-Suc] ion at *m/z* 1363, was also observed, as shown in Table 1 and Figure 2e and f.

These observations contrast strikingly with the collision-induced dissociation (CID) ES mass spectrum of the alkylated octasaccharide generated by the neutral methylation of the octameric oligosaccharide repeating unit of the HMWS, in which CID of methylated and alkali metal-cationized oligosaccharides yielded a combination of glycosidic and cross-ring cleavages.[12] From the ESI-CAD mass spectra observed in this study, interestingly, the acetyl and the pyruvyl substituents within all the monomers were not released even after a CE of 70 eV was applied, indicating that the acetyl and the pyruvyl substituents were more stable than the succinyl modifications in the octasaccharides. Furthermore, no ring-opening fragments of the hexose residues of the octasaccharides occurred at the CEs applied in this study.

In conclusion, the present study showed that the deprotonated ions of the intact and non-methylated *Sinorhizobial* succinoglycan monomers in the negative ion mode were more stable than those of the neutral methylated and sodium adduct of the octamer repeating unit which were previously reported,[12] thereby giving rise to mild ionization of the succinoglycan monomers. Upon ESI-CAD, the greater stability of the acetyl and the pyruvyl ester linkages than the succinyl within the monomers might be attributed to the differences in the hydrogen-bonding networks in which the acetyl or the pyruvyl group may form stronger intra-molecular hydrogen bonds with the carbohydrate moieties than the succinyl groups. The results would be helpful to study the stability of the substituents within hexose residues or the stability of glycosidic bonds using ESI-CAD tandem MS through the product ion scan mode in oligosaccharides modified with anionic substituents.

1. Experimental

1.1. Preparation of the succinoglycan monomers

The monomers were prepared as described in the previous report,[16] and then the purified monomers were confirmed with NMR spectroscopy.

1.2. Mass spectrometric analysis

The monomers were dissolved in a 1:1 solution of water and MeOH and directly infused into the ESI source at a rate of 1 mL/min. The low-energy CAD experiments using N_2 as collision gas were carried out on an API 4000™ triple quadrupole LC/MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a turbo ESI source. The ESI mass spectra in the negative ion mode were measured at a scan range from *m/z* 100 to 1700 with a spray voltage of −4.5 keV in product ion scan mode (MS2) that provides structural information on the fragments of the original ions. The applied CEs were −25, −50, and −70 eV.

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